Development of a Chlamydia trachomatis neutralization assay

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Introduction
There is evidence supporting an important role for neutralizing antibodies in the protection against C. trachomatis infections. The classical in vitro neutralization assays using immunofluorescent staining are laborious, subject to investigator bias and not useful for large scale screening. We therefore developed a neutralization assay which is based on an automated method for enumeration of inclusion forming units (IFUs) using a 96-well plate format as described by Wang et al., 2007.

Methods
The human cervical epithelial cell lines, HeLa and ME-180 are used for infection with C. trachomatis. After 2 days of infection the cells are fixed and IFUs are detected with an anti-C. trachomatis LPS monoclonal antibody, followed by secondary antibody binding, using biotin-conjugated goat-anti-mouse IgG followed by incubation with streptavidin-poly-HRP. The plates are developed with a colorimetric reaction, using TMB reagent. The IFUs are counted with an AEVIS ELISPOT reader. For neutralization ME-180 cells are used and prior to infection bacteria are incubated with 2 fold dilutions of sera from a C. trachomatis infected pig prior to infection.

Results
After two days of infection, IFUs could be detected with specific antibodies, while the negative controls (non-infected), treated with the same antibodies, revealed no spots (Figure 1). IFUs could easily be calculated by titrating a C. trachomatis containing sample in a 96-well plate with a monolayer of HeLa or ME-180 cells. After enumeration of the number of IFUs, the margins can be drawn to reliably calculate the number of spots/µl sample (Figure 2). The pig model is a relatively new but promising model to study vaccine efficacy. We therefore decided to use serum from a Chlamydia infected pig to develop this neutralization assay. Neutralizing activity was clearly demonstrated in three independent experiments (Figure 3).

Figure 1. Clear spots corresponding to C. trachomatis inclusions in fixed cells are visible on the bottom of the wells of a white 96-well plate after the colorimetric reaction with TMB substrate.

Figure 2. The samples are titrated in a 96-well plate, after two days of infection the plate is developed and the spots enumerated with an AEVIS ELISPOT reader. The number of IFUs in this example can be calculated with dilutions 8 to 11 (as indicated with red lines).

Figure 3. Neutralization was measured in three independently performed neutralization tests with different numbers of IFUs per well using serum from a C. trachomatis infected pig. A serum from a non-infected pig (A, B) or a sample without serum (C) was included and averaged to calculate the percentage neutralization (D, E, and F). In test 1 and 2 the serum was adsorbed with kaolin to remove serum components that could interfere with neutralization.

Conclusion
• The automated method for enumeration of inclusion forming units (or IFUSPOT) is a convenient and useful method to determine the number of infectious elementary bodies.
• We have demonstrated that this IFUSPOT assay can be used to study neutralizing activity of sera from infected animals.
• This assay will support the discovery of antigens aimed at the development of a C. trachomatis vaccine.

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References